

Attorney Docket No.: DC-0199
Inventors: Cheung et al.
Serial No.: 10/043,539
Filing Date: January 11, 2002
Page 2

In the Specification:

Please replace the paragraph beginning at page 1, line 8, with the following rewritten paragraph:

-- The present invention relates generally to the field of molecular biology. More particularly, certain embodiments concern methods and compositions comprising DNA segments and protein derived from ~~Staphylococcus aureus~~ Staphylococcus aureus and other bacterial species. The present invention also relates to the three-dimensional structure of proteins derived from *S.aureus* and other bacterial species and methods of identifying and developing pharmaceuticals using, among other things, drug screening assays.--

Please replace the paragraph beginning at page 2, line 13, with the following rewritten paragraph:

-- *S.aureus* can cause a wide spectrum of infections ranging from superficial abscesses, pneumonia and endocarditis to sepsis (4). The ability of *S.aureus* to cause a multitude of human infections is due in part to an impressive array of extracellular and cell-wall associated virulence determinants that are coordinately expressed in this organism (51). The coordinate expression of many of these virulence determinants in *S.aureus* and other bacteria is regulated by global regulatory elements such as *sarA* (staphylococcal accessory regulatory protein A) and *agr* (15, 34). These regulatory elements in turn control the transcription of a wide variety of unlinked genes many of which have been implicated in pathogenesis.--

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Please replace the paragraph beginning at page 3, line 27, with the following rewritten paragraph:

-- The present invention provides a new genetic locus of *S.aureus* and other bacteria. The gene at this locus is referred to herein as *sarR* (staphylococcal accessory regulatory protein R). The *sarR* gene is involved in the regulation and expression of virulence determinants in *S.aureus* and other bacteria. --

Please replace the paragraph beginning at page 22, line 11, with the following rewritten paragraph:

-- The activities of *sarA* promoter fragments linked to the *gfp_{uvr}* reporter gene in RN6390 and its isogenic *sarR* mutant were assayed by flow cytometry. Bacterial cell suspensions obtained at different parts of the growth cycle were analyzed in a ~~FACscan~~ FACSCAN cytometer (Becton Dickinson, Franklin Lakes, NJ). After filtering bacterial samples through a ~~5µm~~ 5 micron filter to remove large aggregates, bacteria were detected by side scatter data as described by Russo-Marie et al. (56). Fluorescence and side scatter data were collected with logarithmic amplifiers. The fluorescence data were reported in fluorescence units as specified by the instrument (~~FACscan~~ FACSCAN cytometer).--

Please replace the paragraph beginning at page 26, line 1, with the following rewritten paragraph:

-- **Over-expression of SarR and production of monoclonal antibodies:** To obtain a large amount of *SarR*, the *sarR* gene was cloned into pET11b and the gene product was over-expressed under an IPTG-inducible promoter in *E.coli* BL21. The expression,

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purification and the purity of the *SarR* protein are shown in Fig. 2. Equivalent volumes of protein fractions during the purification process was applied to a 12% SDS-polyacrylamide gel.

Fig. 2, Lane 1, whole cell lysate of *E. coli* containing pALC1357 (pET11b with the *sarR* gene); Fig. 2, lane 2, supernatant of the cell lysate after clarification by centrifugation; Fig. 2, lane 3, supernatant before 40% ammonium sulfate precipitation; Fig. 2, lane 4, pellet resulting from 40% ammonium sulfate precipitation; Fig. 2, lane 5, pellet from 80% ammonium sulfate precipitation; Fig. 2, lane 6, fall through of the redissolved 80% ammonium sulfate precipitant as applied to a ~~MonoQ~~ MONOQ anion exchange column (Pharmacia); Fig 2, lane 7, fall through from the ~~MonoS~~ MONOS cation exchange column (Pharmacia); Figure 2, lane 8, NaCl elution from the ~~MonoS~~ MONOS cation exchange column. N-terminal sequencing confirmed the identity of the purified *SarR* protein. The *SarR* protein was expressed primarily in the cytosolic fraction (Fig. 2, lane 2). After 80% ammonium sulfate precipitation (Fig.2, lane 5), the redissolved proteins were dialyzed and applied to an anion exchange column (RESOURCE-Q, Pharmacia), only to be found in the fall-through (Fig. 2, lane 6). The flow-through was then applied to a cation exchange column (RESOURCE-S column, Pharmacia) and eluted with a salt gradient. Using this purification scheme, *SarR* was purified to near homogeneity (Fig. 2, lane 8). The authenticity of *SarR*, was confirmed by N-terminal sequencing. The purified *SarR* was then used to immunize mice for the production of anti-*SarR* monoclonal antibodies. Three monoclonal antibodies, designated 2A7, 2C7, and 5E4 were obtained. Despite the similarity between *SarR* and

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SarA, cross-reactive studies indicated that anti-SarR monoclonal antibodies only reacted with SarR and not SarA on immunoblots. --